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A New Technique to Monitor Larval and Juvenile Salamanders in Stream Habitats

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INTRODUCTION

Concern over amphibian population declines and species extinctions (e.g., Blaustein & Wake, 1990; Wyman, 1990; Wake, 1991; Green, 1997; Lannoo, 1998) first surfaced in the late 1980s. It has since generated efforts to determine the status of these animals around the world. Early reviews of the scientific literature and known ongoing studies demonstrated that too few long-term studies were available to assess trends for amphibians in any region (Pechmann et al., 1991; Vial & Saylor, 1993; Mitchell et al., in press). This resulted in a call by the scientific community for the establishment of long-term studies using standardized techniques so that comparisons could be made across space and time. The survey and monitoring protocols outlined in such publications as Heyer et al. (1994), Fellers & Freel (1995), and Olson et al. (1997) have been since utilized in studies around the world. The techniques that may be utilized on streamside salamanders, such as quadrat sampling, transect sampling, and visual encounter surveys, assume that hand capture methods will be used, a method best suited for adults. Juvenile and larval salamanders of species inhabiting stream habitats, however, pose special problems with regard to catchability and handling.

Juveniles and larvae are particularly difficult to capture by hand because of their wet, slippery skin. In addition, these small animals are adept at escaping into numerous interstices among the rocks and gravel in and along the sides of streams. Capturing salamanders in these hiding places requires researchers to disturb microhabitats in small, fragile first- and second-order streams by moving rocks and digging into the substrate. We describe herein the use of refugia bags as a technique to capture and monitor juvenile and larval salamanders in streams without disturbing the habitat. We also provide results from two studies to demonstrate the effectiveness of this methodology for selected species of streamside salamanders in the Appalachian Mountain region.

MATERIALS AND METHODS

We designed refugia bags while we were conducting studies on the life history of the seal salamander (*Desmognathus monticola*), a common streamside salamander in the Appalachian Mountains (Petranka, 1998). The idea to use refugia bags for amphibians resulted from the work of aquatic invertebrate biologists at the Fernow Experimental Forest lab who reported finding juvenile and larval salamanders in leaf packs similar to those described in Merritt & Cummins (1996). Juvenile and larval salamander refugia bags (Fig. 1) were constructed from plastic netting with a mesh size of 3-4 cm. The netting is the type used to cover trees to protect fruit from birds and can be ordered from forestry supply companies or purchased at feed and farm stores and garden centers. Netting comes in rolls or in flat, folded sections. The folded netting is easier to lay out, measure, and cut. Netting should be cut into 45-50 x 30 cm sections, and small rocks (no larger than 10 x 15 cm) placed in the bottom. Layers of mosses or leaves should be placed on top of the rocks. Pull the tops of the sections tightly up and around the rocks and leaves, and tie off the top with twist-ties. If the bag is not tied tightly, then adult salamanders may also seek shelter in the leaves. Bags should be placed in water in the streams but not submerged. Orange or red flagging can be tied to each bag for easy recognition in the field. This is especially important in the event that high water covers the bag with debris. To prevent bags from washing downstream during high water events, place a large rock on the bag or place two large rocks below (and touching) the bag. Bags can be checked periodically as dictated by the study design. A bag is checked by lifting it from the stream and quickly placing it in a white plastic dishpan and shaking it until all salamanders have emerged from the bag into the dishpan. The pan provides a white background against which the salamanders can be easily observed. The bag can be returned to the desired location in the stream.

Once caught, juveniles can be identified to species,

Fig. 1. Example of a completed refugia bag used to capture larval and juvenile streamside salamanders.

counted, measured for total length, snout-to-vent length, and released. Data can be recorded as the number of salamanders of each species and age class per refugia bag per sampling time interval or per sampling area. Data from refugia bags provide estimates of relative abundance among sites and species when compared under similar environmental conditions and over similar sampling periods.

Refugia bags are similar to leaf pack samplers and basket samplers used by aquatic entomologists to study macroinvertebrates in streams (e.g., Anderson & Mason, 1968; Hilsenhoff, 1969; Crossman & Cairns, 1974; Petersen & Cummins, 1974). Leaf packs are used to sample larval insects in riffles of streams or small rivers and basket samplers are cylindrical wire baskets packed with rocks and leaves for use in riffles of larger rivers. Both techniques are used for qualitative and semi-quantitative sampling (relative abundance), rather than strict quantitative sampling to density values (Merritt & Cummins, 1996).

RESULTS AND DISCUSSION

We used refugia bags initially during 1989-1994 in five watersheds in the Fernow Experimental Forest, USDA Forest Service Northeastern Research Station at Parsons, West Virginia. Of the 1,523 juvenile salamanders captured during this period, 66.5% were captured in bags and 33.5% were captured by hand after turning rocks.

We also examined the effectiveness of refugia bags in two studies of juvenile and larval salamanders in first-order streams in eastern West Virginia from 1996 to 1998. We conducted the first study in four streams on the Westvaco Wildlife and Ecosystem Research Forest in Randolph County and the second study in nine streams on the U.S. Department of Agriculture gypsy moth control study plots in the Monongahela National Forest in Pocahontas County. In these studies, seven streams were spring fed and maintained a constant flow of water. Six streams had no spring connections and had intermittent flows.

We found five species of salamanders in the 13 first-order streams in both studies. Of these, *Desmognathus* juveniles used the bags more frequently than *Gyrinophilus porphyriticus* and *Eurycea bislineata* juveniles (94 vs 7), but larvae of the latter two species occupied the bags more often than *Desmognathus* larvae (131 vs 24) (Table 1). The low number of *Desmognathus* larvae is probably due to the short larval periods and restricted habitat of these species in the Appalachian region (Petranka, 1998). The larval period for *Desmognathus ochrophaeus* is known to be less than two weeks, and in some cases there is no free-swimming larval stage (Marcum, 1994). Newly hatched *D. monticola* larvae stay in water film on the undersides of rocks, and at later developmental stages inhabit the interstices of the substrate in riffles (Marcam, 1994), making it less likely that they will encounter the bags.

We also evaluated the effectiveness of refugia bags in relation to capturing salamander larvae with aquarium nets and searching the bottoms of streams at night with flashlights. Data in Table 2 demonstrate that, with the exception of larvae that inhabit plunge pools, such as *E. bislineata*, bags are at least as effective as using nets and more effective than night searches. Night searches and refugia bags are less disruptive to the stream habitat than searching with nets, and bags are more useful when daytime searches are necessary.

Several effective techniques have been used by investigators to capture salamander larvae (Shaffer et al., 1994; Fellers & Freel, 1995; Mitchell, 1998a, b). These techniques involve using a tea strainer or aquarium dip net to capture larvae from silt in plunge pools or interstices in the substrate. Although larvae can be captured in pools without disturbing the substrate, it is usually necessary to dig into the rocks and gravel with a tea strainer to capture small larvae. Refugia bags are effective for salamanders because they provide a refuge where juveniles can find shelter from cannibalistic adults and predatory species. A primary assumption with this technique is that the escape behaviors of juveniles and larvae of all species sampled are similar. Refugia bags may also provide a substrate for small food items for juveniles, although this needs further investigation. The use of refugia bags provides a non-intrusive method for collecting and monitoring juvenile and larval streamside salamanders without disturbing the substrate. This microhabitat is used by various life history stages of small benthic invertebrates and is also where the eggs and larvae of several species of salamanders reside. Refugia bags provide an effective method for studies on the ecology and life histories of streamside salamanders, and they are effective tools for use in long-term monitoring programs.

Table 1. Total number of juveniles and larvae of five species of salamanders captured in refugia bags in first-order streams in the Westvaco Wildlife and Ecosystem Research Forest (1996-1997) and the USDA gypsy moth study plots in eastern West Virginia (1997-1998). Total number of trap days was 274; each bag was checked monthly June-October.

Species	Larvae	Juveniles	Total
Desmognathus fuscus	11	25	36
Desmognathus monticola	12	46	58
Desmognathus ochrophaeus	1	23	24
Eurycea bislineata	51	5	56
Gyrinophilus porphyriticus	80	2	82

Table 2. Number of larval salamanders captured by three different collection methods in first-order streams in USDA gypsy moth study plots in eastern West Virginia. Number of trap days for bags = 274 (monthly, May-September) and number of sampling periods for nets and night searches = 12 (monthly, June-September 1997 and 1998).

Species	Refugia Bags	Aquarium Nets	Night Searches
Desmognathus fuscus	11	13	1
Desmognathus monticola	6	3	1
Desmognathus ochrophaeus	1	3	0
Eurycea bislineata	22	333*	15
Gyrinophilus porphyriticus	22	28	7
Total	62	380	24

* Number of *E. bislineata* is high because all searches in 1998 were conducted in pools where this species was abundant.

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